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Biodegradation of starch–graft–polystyrene and starch–graft––poly(methacrylic acid) copolymers in model river water

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Abstract: In this paper, a biodegradation study of grafted copolymers of cornstarch and polystyrene (PS) and cornstarch and poly(methacrylic acid) in model river water is described. These copolymers were obtained in the presence of different amine activators. The synthesized copolymers and products of degradation were characterized by Fourier transform infrared (FTIR) spectroscopy and scanning electron microscopy (SEM). Biodegradation was monitored by mass decrease and the number of microorganisms by the Koch method. Biodegradation of both copolymers advanced with time, the starch––*graft*–poly(methacrylic acid) copolymers had completely degraded after 21 day, and the starch–*graft*–polystyrene had partially degraded (45.8–93.1 % mass loss) after 27 days. The differences in the degree of biodegradation are the consequences of the different structures of the samples, and there was a significant negative correlation between the share of polystyrene in the copolymer and the degree of biodegradation. The grafting degree of PS necessary to prevent biodegradation was 54 %. Based on experimental evidence, the mechanisms of both biodegradation processes are proposed, and influence of degree of starch and synthetic component of copolymers on the degradation of the samples were established.

Keywords: graft copolymers; model river water; degradation study; statistical analysis.

INTRODUCTION

The problem of the accumulation of synthetic polymeric materials in the environment is not only attracting growing attention worldwide, but also indi-

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cates the requirement for producing new, alternative, biodegradable materials with high levels of biodegradability that could, in the future, replace the bulk polymers of today. Conventional polymeric materials are usually not degradable or biodegradable at measurable or significant rates under environmental conditions. For this reason, the search for polymers that could be biodegradable under environmental conditions, such as copolymers containing biodegradable component and conventional, non-biodegradable component, has become the focus of academic and industrial communities.[1]

Polystyrene (PS) and poly(methacrylic acid) (PMAA) represent two of the major "targets" for preparation of their biodegradable derivatives. Polystyrene has excellent physical properties, low price and is easy to process, while PMAA is an adaptable macromolecule that offers many benefits in the development of new materials for a host of applications. Modification/functionalization of these polymers to the extent where they will become submissive to microbial attack is one of the alternatives to overcome environmental problems. Modifications with natural polymers, such as carbohydrates, represent major techniques in the creation of more biodegradable polymers.[2–6] Gelatin,[7] natural rubber,[8] lignin[9] and other reactants can also be used for such a purpose. One of the ways biodegradable derivates of polymers can be obtained is through grafting reactions. Grafted copolymers of polystyrene and poly(methacrylic acid) with starch (starch–graft––PS and starch–graft–PMAA) can be obtained in different ways: by suspension[10] and emulsion polymerization,[11] or by ^{60}Co-radiation.[8,12] In all the mentioned grafting reactions, different initiating system can be used.[13–21] In investigation of degradation and biodegradation processes, different methods such as UV degradation, degradation in soil, degradation by different types of microorganisms, biodegradation in natural and waste water, *etc*. are being used.[22–27]

Degradation in river water was used for the study of biodegradation of surfactants,[28] such as linear alkyl benzene sulfonates (LAS),[29] bisphenol A[30,31] and various glycols.[32] Moreover, great attention has been paid to the degradation processes of chlorinated aliphatic hydrocarbons, such as 1,2-dichlorethane,[33] in three European rivers (the Ebro, Elbe and Danube) and polycyclic aromatic hydrocarbons (PAH) in simulated nuclear wastewater.[34] Biodegradations of synthetic polymers, such as fibers for drift net from poly(3-hydroxyalkanoic acids) in a reactor with different types of nature waters (river, sea and lake waters),[35] vinyl polymers, such as polyethylene in river water,[36] its blend and copolymers with natural polymers (in activated sludge),[37] poly(ethylene glycols) (PEGs)[38] and different polyurethanes[39] (both in sea water) were also studied.

Polystyrene is used for housings for TVs, refrigerators, air conditioners, microwaves, blenders, protect boxes for DVDs and all kinds of IT equipment where the criteria for use are combinations of function, form and esthetics and high performance/cost ratio. Starch–graft–PS copolymers were synthesized in order to

obtain new plastics with similar or better characteristic than pure polystyrene and to reduce environmental pollution caused by polystyrene. Potential applications for starch–graft–PS copolymers are in the textile industry (this copolymer increases the tensile strength of cotton yarn),[40] as superabsorbent[41] and as surface-sizing agents.[42] Potential applications for starch–graft–PMAA copolymers are in the textile industry (as a sizing agent that increases abrasion resistance compared to starch),[20] in waste water treatment for removal of dyes,[43,44] and hydrogels of this copolymer can be used as a matrix for drug delivery.[45,46]

In the present study, the biodegradation of starch–graft–polystyrene and starch–graft–poly(methacrylic acid) copolymers synthesized in aqueous medium with potassium persulfate (PPS) as an initiator and different amines as polymerization activators were investigated. To the best of our knowledge, no previous literature references describe the biodegradation of these graft copolymers in a river water system. A continuous flow system with recirculation, enabling the simulation of environmental conditions in the laboratory, was applied in the biodegradation study, while the biodegradation medium was model water of Sava River, an international river and one of the main tributaries of the Danube.

EXPERIMENTAL

Materials

The cornstarch (Centrohem, Serbia) used in the grafting experiments was dried for 3 days under vacuum at 50 °C, before use. Styrene (Aldrich Chemicals) was purified by distillation under reduced pressure prior to polymerization, while methacrylic acid (Fluka) was used as obtained. Potassium persulfate (PPS) (Aldrich Chemicals), sodium dodecyl sulfate (SDS) (Aldrich Chemicals), methanol (Fluka), chloroform (J. T. Baker), and amines – *N*,*N*-dimethylethanolamine, *N*,*N*-diethylethanolamine, triethylamine, *n*-propylamine, isobutylamine, *n*-pentylamine *n*-hexylamine, 1-(2-hydroxyethyl) piperazine and 4-(2-hydroxyethyl) morpholine (all Fluka) were used as obtained.

Water from the Sava River was taken from a location near the riverbank, 2 km before the confluence of the Sava and Danube Rivers. 2 L of this water were mixed with artificial wastewater, containing 800 mg of peptone, 550 mg of meat extract, 150 mg of urea, 35 mg of NaCl, 20 mg of $CaCl_2 \cdot 2H_2O$, 10 mg of $MgSO_4 \cdot 7H_2O$ and 140 mg K_2HPO_4 in 3 L of distilled water, thus making total of 5 L of model river water of pH 7.5.

Methods

Starch–graft–PS samples and starch–graft–PMAA samples used in biodegradation experiments were prepared in the laboratory prior to the experiments. Synthesis of starch–graft––PMAA copolymers and the obtained results were previously described.[47] The starch–graft–PS copolymers were prepared by emulsion polymerization in the presence of potassium persulfate/amine initiation redox system and sodium dodecyl sulfate (SDS) as the emulsifier. Water (80 mL), cornstarch (10 g), styrene (10 g) and SDS (0.23 g, 8.0×10^{-4} mol) were first added in the reaction vessel. The components were homogenized and heated to the reaction temperature of 75 °C. After reaching the reaction temperature, the initiator potassium persulfate (0.40 g, 0.0015 mol) in 20 ml of distilled water was added followed by the amine activator. Samples with different percentages of grafting were obtained using different amines

(*N*,*N*-dimethylethanolamine, *N*,*N*-diethylethanolamine, triethylamine, *n*-propylamine, isobutylamine, *n*-pentylamine *n*-hexylamine, 1-(2-hydroxyethyl) piperazine and 4-(2-hydroxyethyl) morpholine) or different amounts of the same amine (*N*,*N*-dimethylethanolamine in amount of 0.001, 0.002 and 0.004 mol). In all syntheses, the reaction mixture was stirred at 275 rpm, while the reaction time was 15 min, to prevent starch gelation. One reaction was performed under the same reaction conditions but without an amine activator. This reaction was used to evaluate the influence of the amine activator on percentage of grafting. Non-polymerized styrene monomer was removed by extraction in methanol for 24 h. In order to remove unbound polystyrene homopolymer, extraction in chloroform was performed, and the unbound polystyrene was then precipitated with methanol. In order to determine the amount of bound polystyrene and the percentage of grafted polystyrene (*G* / %) in the starch–graft–PS copolymer, the samples were hydrolyzed using 100 mL of 1 M HCl for 5 g of copolymer. The hydrolysis was performed for 2 h at 200 °C. After hydrolysis, non-hydrolyzed polystyrene remained (confirmed by FTIR spectroscopy) in the form of a solid residue which was filtered, rinsed with distilled water and dried at 50 °C to constant mass. Percentage of grafting was calculated by the following formula:

$$G = 100 \frac{m \text{ of PS after hydrolysis}}{m \text{ of copolymer before hydrolysis}} \quad (1)$$

In this investigation, graft copolymers of polystyrene and poly(methacrylic acid) with starch with maximal percentage of grafting were obtained. The targeted values for the percentage of grafting were 30 % (which is one of the highest values in the literature to date) or higher. For both types of copolymers, this value was achieved (32.5 % for starch–graft–PS and 40.9 % for starch–graft–PMAA copolymer).

From the sum of the mass of polystyrene that was extracted in chloroform, the mass of polystyrene after hydrolysis and mass of the styrene monomer, the yield of polystyrene (*Y)* could be calculated in the grafting reaction from the following formula:

$$Y / \% = 100 \frac{m \text{ of PS}}{m \text{ of styrene monomer}} \quad (2)$$

Each synthesis was performed three times in order to monitor the reproducibility of the results. Deviations in the grafting percent were very small, amounting to less than 2 %. The obtained grafting percent of the starch–graft–PS copolymers was one of the highest ever registered in the literature.

Eleven samples of starch–graft–PS copolymers, ten samples of starch–graft–PMAA copolymers synthesized with or without amine activator, and three control samples (pure starch, polystyrene and poly(methacrylic acid)) were subjected to the biodegradation process. All the copolymer samples contained different percentages of grafting. Copolymers with similar percentages of grafting were synthesized using different amine activators, and they were studied as different samples. Hence, samples obtained using different amine activators had different structures which affected the biodegradation results. Before biodegradation, the samples were milled and pressed in the form of discs with a diameter of 1 cm and thickness of 0.2 cm. Biodegradation of starch–graft–PS copolymers was performed separately from the biodegradation of the starch–graft–PMAA copolymers.

Different types of microorganisms were isolated in the water from the Sava River, such as *Escherichia coli*, *Proteus* sp., *Serratia marcescens*, *Klebsiella* sp., *Pseudomonas aeruginosa* and *Enterococcus faecalis*. The number of microorganisms was monitored by the Koch

method. This method determines the number of living cells indirectly, usually after cultivation. After incubation on a solid medium (agar) in Petri dishes, the colonies were counted with the assumption that each cell created one colony. This method provides a realistic number of cells, but its duration is long (at least 24 h). The number of microorganisms was expressed as log (*CFU* / mL^{-1}), where *CFU* stands for colony forming units and is expressed per mL of the model river water.

Characterization of the copolymers before and after biodegradation

Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM) were used before (to prove successful grafting) and after biodegradation (to confirm changes caused due to biodegradation) as methods for characterization of graft copolymers. The FTIR spectra were recorded from KBr discs on a MB-100 Bomem FTIR spectrophotometer. For SEM, the samples were mounted on copper and metallized with gold–palladium using a Polaron SC 502 sputter coater and recorded on a Jeol JSM 5800 scanning electron microscope with the filament operating at 20 keV.

Laboratory model of systems for biodegradation

Biodegradation of the starch–graft–PS and starch–graft–PMAA copolymers by natural microorganisms in model river water was performed at room temperature by fractional elution during 21 days (for starch–graft–PMAA copolymers) and 27 days (for starch–graft–PS copolymers). The employed apparatus, consisting of a reservoir for river water (A), a dosing pump (B), an aeration vessel (C), a sample storage vessel (D), a recirculation pump (E), an effluent collection vessel (F), an aeration pump (G) and flow meter (H), is shown schematically in Fig. 1.

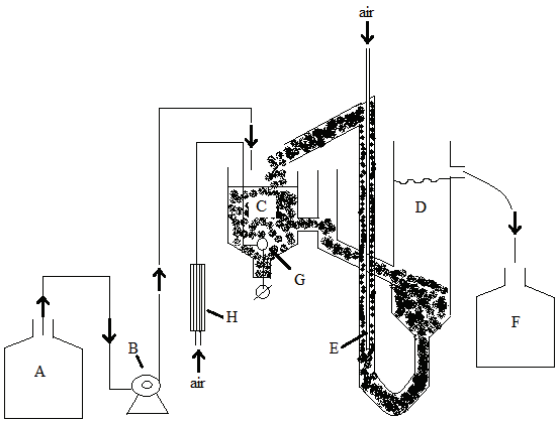

Fig. 1. Apparatus for the determination of the degree of biodegradation.

Biodegradation procedure

Five liters of model wastewater were added in vessel (A). Aeration vessel (C) and then sample storage vessel (D) were filled with water from vessel (A) using the dosing pump (B). When vessel D had been filled, the aeration pump (G) and the recirculation pump (E) were turned on. The aeration lasted for 48 h, until the microorganisms had adapted to the new conditions. The dosing pump (B) was set to minimum flow, conditions that could be considered as stationary. From time to time, it was necessary to empty the effluent collection vessel (F) by transfer of the effluent to vessel (A). After 48 h of recirculation, samples of copolymers

were added to the system, in column (D). All the 24 samples were put in a mesh, water permeable bag and after that, all samples of the same type of copolymer were put in a big, water permeable sack and sunk to the middle of column (D). The biodegradation experiments were performed separately. The biodegradation of the starch–graft–PS copolymers was the first to be monitored. After this experiment, the system was emptied, then cleaned and dried. A new amount of model wastewater (5 L) was made and the whole process was repeated using the starch–graft–PMAA copolymers.

The water flow was kept constant at 2 L day^{-1}. The samples were removed every 9 days when the starch–graft–PS copolymers were used and every 7 days for the starch–graft–PMAA copolymers. After removal, the samples were rinsed with distilled water and dried to constant weight under vacuum at 50 °C for 24 h. During drying of the samples under vacuum, the biodegradation test was not interrupted. After drying, the samples were returned to the aqueous system and the same samples were again exposed to the microorganisms. The percentage of biodegradation was calculated based on the following formula:

$$\text{Biodegradation} = 100\frac{(m_0 - m_x)}{m_0} \quad (3)$$

where m_0 is the sample mass before biodegradation and m_x is the sample mass after removal from model wastewater.

RESULTS AND DISCUSSION

Characterization of starch–graft–*PS and starch*–graft–*PMAA copolymers*

FTIR spectroscopy was used to verify that grafting had occurred. For starch––graft–PS copolymers, the characteristic peaks were: 3020–3080 (C–H vibrations of the aromatic ring), 2000–1660 (C–C vibrations in the benzene ring), 1490, 760 and 700 cm^{-1} (C–H stretching of the aromatic ring), originating from the polystyrene moieties, and 3000–3600 (O–H stretching), 2880–2920 (C–H stretching), 1645 (O–H first overtone) and 1190–950 cm^{-1} (C–O stretching), originating from the starch. The presence of these peaks indicated that the grafting was successful (Fig. 2).

The FTIR spectrum of the starch–graft–PMAA copolymer and its characteristic peaks were previously shown and described.[48]

The FTIR spectra of starch–graft–PS copolymers were also recorded after the biodegradation test. The FTIR spectrum of the copolymer with a grafting percentage of 12.9 % (sample 2) is shown in Fig. 3a together with the FTIR spectrum of pure polystyrene (Fig. 3b). It can be seen that these spectrums are identical and all the characteristic peaks of pure polystyrene are present in the spectrum of the copolymer after biodegradation. During biodegradation test, only starch was subjected to biodegradation, while no degradation of polystyrene occurred. At the end of the biodegradation process, only polystyrene remained and in case of sample 2, it can be concluded that the starch initially present was completely biodegraded.

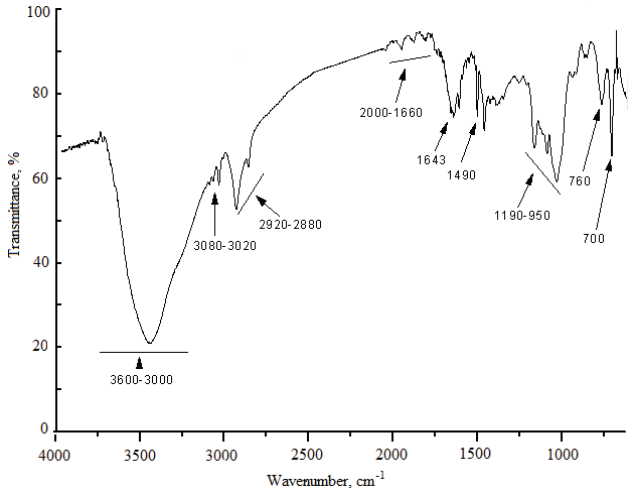

Fig. 2. FTIR Spectrum of starch–graft–PS with a grafting percentage of 20.9 %.

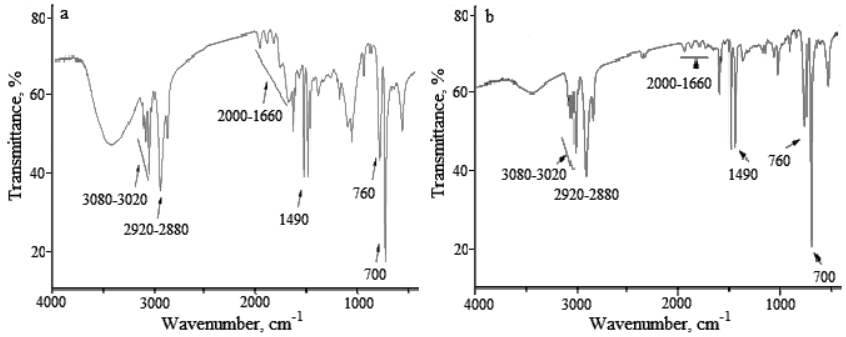

Fig. 3. FTIR Spectra of a) starch–graft–PS copolymer with a grafting percentage of 12.9 % after 27 days of the biodegradation test and b) pure polystyrene.

The FTIR spectra of the starch–graft–PMAA copolymers were not recorded after the biodegradation test because in all samples, as well as complete degradation of starch, complete dissolution of PMAA occurred.

SEM micrographs of grafted copolymers starch–graft–PS with different *G* are shown in Fig. 4. The structure of the starch–graft–PS copolymer in which the polystyrene links to starch granules (Sample 3, lower percentage of grafting) is depicted in Fig. 4a. This network structure does not cover the whole surface, thus the granules of starch are easily visible. Figure 4b (Sample 6) shows the sample of starch–graft–PS with a higher percentage of grafting, where polystyrene adheres to almost all the starch granules. Fig. 4c represents the SEM micrograph of pure cornstarch (starch used in grafting reaction). On comparing the SEM micrographs, it could be stated that there was a change in the granular shape of starch before and after copolymerization. When cornstarch granules are heated in the

presence of water, they undergo changes in size and shape. The granules initially swell and take the shape of a flattened disk. As the heating continues, the granules take on a puckered appearance.[48]

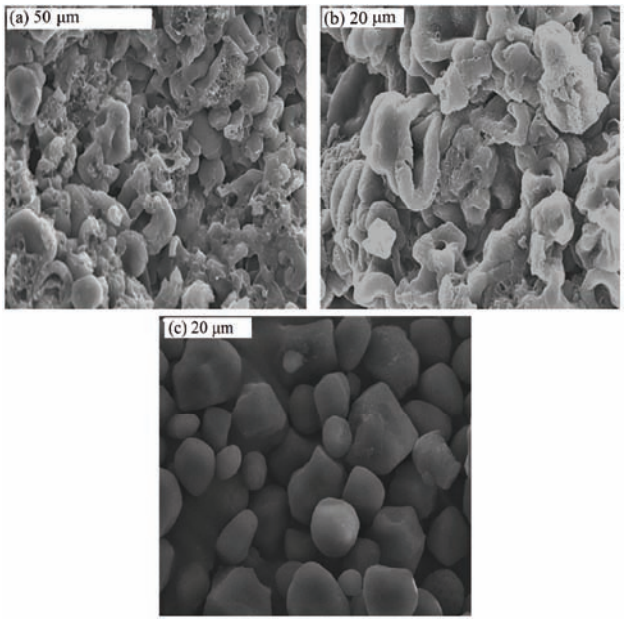

Fig. 4. SEM Micrographs of starch–graft–PS copolymers with percentages of grafting of a) 16.2, b) 25.3 % and c) of pure corn starch.

SEM Micrographs of starch–graft–PS copolymers (samples 3 and 6) after biodegradation test in model wastewater are shown in Fig. 5, which reveals that only polystyrene particles were present, while no starch granules can be seen. The remaining granules of starch are probably located in the interior and they are completely covered with polystyrene. Based on these micrographs, it could be concluded that biodegradation in the model wastewater truly occurred and that a portion of starch in the copolymer had been degraded.

The SEM microphotographs of starch–graft–PMAA copolymers with different percentage of grafting are shown in Fig. 6. The surface layer of copolymer with a grafting percentage of 22.0 % is shown in Fig 6a, while Fig. 6b presents an intersection of a copolymer with grafting percentage of 40.9 %.

The PMAA in the copolymer was not only present at the sample surface, but also in its interior. In Fig. 6a, it can be seen that starch granules are coming out from plate structure of PMAA and that a large amount of poly(methacrylic acid) can be found inside the sample (Fig. 6b). PMAA nearly completely covers the starch granules, probably in the form of a thin layer, as uncovered parts of the starch granules could be observed in some parts of the microphotograph.

Influence of amine on the initiation reaction

Amines have different abilities to facilitate the decomposition of the initiator. The main reason for using amines lies in decreasing the activation energy required for decomposition of the initiator. The mechanisms of the initiation reactions are different and depend on the type of the amine. If the primary and secondary amines are used, persulfate radical and radical on N-atom of amine molecule initiate grafting reaction, while with the tertiary amines radical center is on the C-atom of the amine component. Steric factors have great influence on the initiation and therefore on the percentage of grafting. During the initiation reaction, one type of complex between potassium persulfate and amine is formed and the size of the group and the alkyl chain lengths that are present on the N-atom of the amine have a great influence on the grafting reaction.

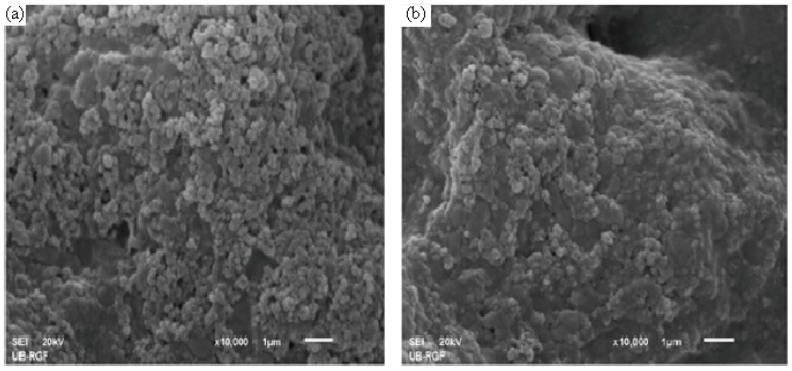

Fig. 5. SEM Micrographs of starch–graft–PS copolymers with percentages of grafting of a) 16.2 and b) 25.3 % after 27 days of biodegradation.

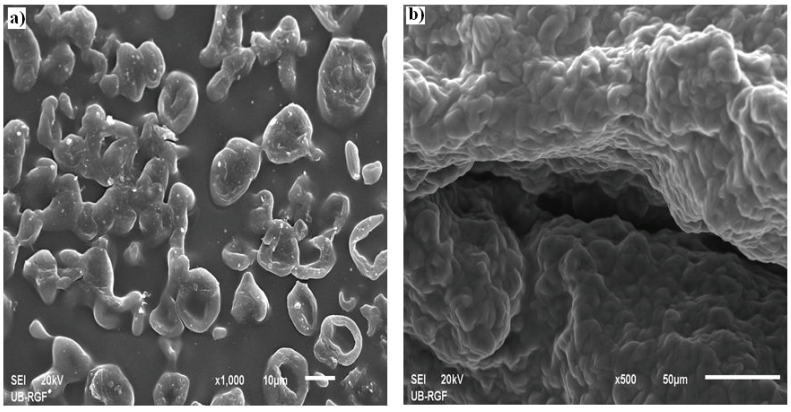

Fig. 6. SEM Micrographs of starch–graft–PMAA copolymers with percentages of grafting of a) 22.0 and b) 40.9 %.

Biodegradation studies

The results of monitoring the degradation of starch–graft–polystyrene and starch–graft–poly(methacrylic acid) copolymers in model river water are given in Tables I and II, which include the results for the negative control (distilled water adjusted to the same pH as the model river water). For starch–graft–PS copolymers in the negative control, the mass loss depended on the amount of starch in the copolymer (the mass loss increased with increasing amount of starch) and varied between 1.7 and 6 %. In the negative control for the starch–graft–PMAA copolymers, the mass loss of starch from the samples was between 1.2 and 4.6 %. These mass losses originated from the solubility of starch and degradation of starch by microorganisms that developed in the water during the experiment.

TABLE I. Monitoring of the degradation of copolymers of starch and polystyrene in model river water compared to the degradation of pure polystyrene and starch

Sample designation	G / %	Y / %	Mass loss after 9 days, %	Mass loss after 18 days, %	Mass loss after 27 days, %	Starch degradation in the samples, %
1	6.9	7.4	62.8	84.3	93.1	100
2	12.9	13.8	51.3	75.6	87.1	100
3	16.2	18.5	35.2	59.3	73.5	87.8
4	18.4	18.3	35.2	52.4	65.1	79.8
5	20.9	23.8	35.8	53.7	66.0	83.5
6	25.3	25.8	22.9	39.2	49.7	66.6
7	25.7	35.3	33.8	51.9	60.9	82.0
8	25.9	36.0	35.3	52.3	61.9	83.5
9	27.3	33.2	22.6	40.0	52.0	71.5
10	28.4	33.3	20.9	36.0	49.1	68.6
11	32.5	43.7	20.5	35.0	45.8	67.9
PS	–	–	0	0	0	–
Starch	–	–	66.8	94.3	100	100

As expected, the degradation processes of both copolymers in model river water advanced with time (Tables I and II). Due to the presence of non-degradable polystyrene, the starch–graft–polystyrene copolymers were not fully degraded after 27 days of the biodegradation process. The final level of degradation (from 45.8 to 93.1 % of total mass or from 66.6 to 100 % of starch mass present in the copolymers) depended on the percentage of grafting and increased with increasing amount of biodegradable starch in the copolymer. This is logical, since polystyrene is non-biodegradable and only the degradation of starch part of the copolymer affected the total biodegradation process. The highest percentages of biodegradation were 93.1 (sample 1) and 87.1 % of the total mass (sample 2) for the two samples with the highest amount of starch in the copolymers. These two samples were the only samples where complete degradation of the starch in the

copolymer occurred (Table I). With decreasing amount of starch in the copolymer, the biodegradation of the starch became incomplete, and this phenomenon could be explained by the different morphologies of the samples. With decreasing amount of starch (increasing of amount of PS) in the copolymer, larger amounts of the starch granules were trapped within the PS chains and thus inaccessible for the microorganisms. Hence, the percentage of biodegradation was smaller than theoretically possible. In addition, the influence of different morphologies could be seen on the example of three copolymers with similar percentages of grafting (samples 6–8). Although the percentages of starch in these samples were approximately the same, the biodegradation results for sample 6 were very different compared to the other two samples (66.6 % for sample 6 compared to 82.0 % for sample 7 and 83.5 % for sample 8), which could be explained by the different structure of the sample 6 compared to those of samples 7 and 8.

TABLE II. Monitoring of the degradation of copolymers of starch and poly(methacrylic acid) in model river water compared to the degradation of pure starch and pure poly(methacrylic acid)

Sample designation	*G* / %	Mass loss after 7 days, %	Mass loss after 14 days, %	Mass loss after 21 days, %
1	8.6	81.04	100	100
2	10.8	48.41	84.2	100
3	13.2	80.89	100	100
4	16.0	59.93	100	100
5	18.5	70.52	100	100
6	21.1	60.82	100	100
7	22.0	67.16	100	100
8	27.0	62.99	96.6	100
9	34.7	42.02	84.7	100
10	40.9	56.65	96.4	100
PMAA	–	100	100	100
Starch	–	39.90	71.0	100

If the percentage of grafting and the percentage of biodegraded copolymer (expressed as percentage mass loss during biodegradation) are correlated, it becomes obvious that there are significant correlations (significance of correlation $p < 0.05$) between these values after 9, 18 and 27 days of degradation (Table III, Fig. 7).

TABLE III. Correlation parameters between the percentage of grafting and the mass loss of starch–graft–polystyrene copolymer samples after 9, 18 and 27 days; number of samples: 11

Correlation parameter	After 9 days	After 18 days	After 27 days
R^a	–0.9210	–0.9419	–0.9596
p^b	0.000	0.000	0.000

[a]coefficient of correlation; [b]significance of correlation

Clearly, the fact that there are significant correlations between the degree of grafting and the degree of biodegradation proves that the level of biodegradation depends on the level of starch in the copolymer, and that, in the present system, the available starch in the starch–graft–PS copolymer degrades completely.

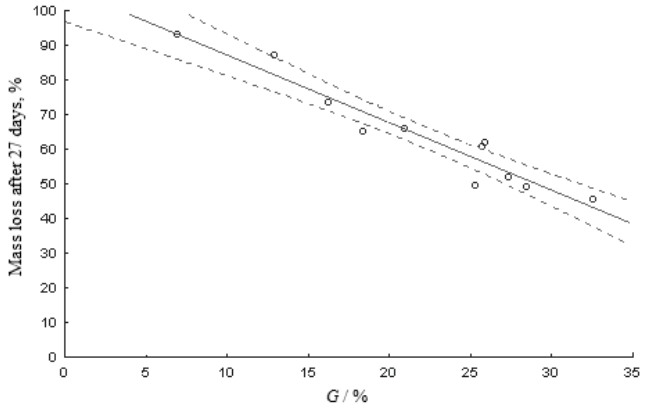

Fig. 7. Linear correlation between the percentage of grafting and mass loss of starch–graft–polystyrene samples after 27 days of biodegradation in model wastewater ($y = 106.70 – 1.952x$).

When the correlation equation is analyzed, it becomes obvious that the grafting degree necessary to prevent biodegradation (the value of percentage of grafting that would result in 0 % biodegradation) was approximately 54 %. Obviously, the biodegradation of the starch–graft–polystyrene copolymer in model river water would be completely suppressed because of the unavailability of microorganisms to degrade starch covered by polystyrene at levels of polystyrene of around 54 % or higher. Since in the examined copolymers, polystyrene was present to much smaller extent (6.9–32.5 %), biodegradation in the model wastewater occurred.

The degradation of starch–graft–PMAA copolymers (Table II) was much simpler than that of the previously discussed starch–graft–PS copolymers, because PMAA is soluble in water. Most of the samples of copolymer completely degraded after 14 days of biodegradation in the model river water, while all of them fully degraded after 21 days. Pure poly(methacrylic acid) completely dissolved after one week, while starch fully degraded after three weeks of the process.

The fact that degradation of starch–graft–poly(methacrylic acid) copolymer is slower than the solubility of pure poly(methacrylic acid) (Table II) probably indicates that the degradation mechanism of copolymer involves two steps: attack of microorganisms on the available starch and, after degradation of available

starch, release of chains of poly(methacrylic acid) that become free and soluble in water.

If the degradation results before completion of degradation, expressed as the percentage of mass loss after 7 days, are correlated with the degree of grafting, there is no dependence. This was expected, hearing in mind not only the fact that one of components is degradable, but also the mechanism of biodegradation proposed above. The rate of biodegradation depended not only on the percentage of grafting, but also on the distribution of starch and poly(methacrylic acid) in the copolymers.

It is also noteworthy that the biodegradation of these copolymers in model wastewater (21 and 27 days) was much faster than their biodegradation in soil samples.[8]

During the biodegradation test in model river water, the number of microorganisms was monitored for both types of copolymers. In case of starch–graft––PS copolymers (Fig. 8), the number of microorganisms increased during the first three days, which is associated with the addition of nutrients that were added before the addition of the samples. After the first three days, the number of microorganisms decreased with decreasing amount of starch in the copolymer to its minimum on the 19th day, when log (CFU / ml^{-1}) = 0. It could be concluded that the amount of pure starch and starch available to microorganisms that remains in copolymers was very small, so the number of formed colonies was zero at a dilution of 10^{-5}. To ensure full degradation after the 19th day, new amount of nutrients was added to the system, which increased the number of formed colonies on the 23th and 27th day. Hence, it could be assumed that the decrease in the number of colonies to zero occurred due to lack of nutrients.

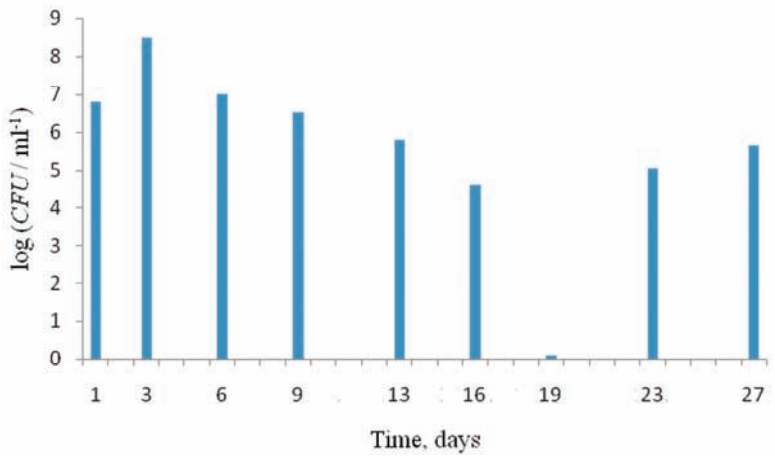

Fig. 8. The change in the number of microorganisms during the biodegradation of starch–graft–PS copolymers.

The number of formed colonies in the system with starch–graft–PMAA copolymers increased during first four days, which is associated with the addition of nutrients (Fig. 9). From the 7th to the 16th day, number of microorganisms was approximately constant (log (*CFU* / ml^{-1}) ≈ 8.5), which indicates sufficient amount of starch in the water. After 16 days, the number of microorganisms decreased and reached a minimum on the 18th day, which indicated the amount of starch in the copolymer had been reduced. To ensure complete degradation, new amounts of nutrients were added to the system, as in case of the starch–graft–PS copolymers.

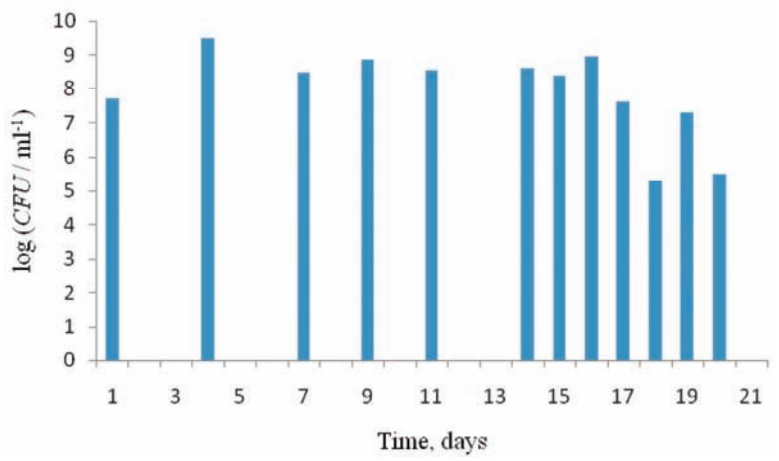

Fig. 9. The change in the number of microorganisms during the biodegradation of starch–graft–PMAA copolymers.

CONCLUSIONS

Graft copolymers of polystyrene and starch and poly(methacrylic acid) and starch, obtained by radical polymerization in the presence of different amine activators, were subjected to biodegradation in model river water. The biodegradation was monitored by the mass decrease of copolymers, FTIR spectroscopy and SEM. The biodegradation of both types of copolymers advanced with time. Starch–graft–poly(methacrylic acid) copolymers were completely degraded after 21 days and the starch–graft–polystyrene copolymers were partially degraded (45.8–93.1 % mass loss) after 27 days. The level of the biodegradation of the starch–graft–PS was significantly negatively correlated with the degree of grafting, *i.e.*, the higher the percentage of polystyrene in copolymer, the lower was the final degree of biodegradation. Only at relatively low levels of polystyrene in the copolymer (above 12.9, but certainly lower than 16.2 %), did all the starch in the copolymer degrade under the investigated conditions. With increasing content of non-degradable polystyrene in the copolymer, some of the starch remained

protected from biodegradation, up to a percentage of polystyrene of around 54 %, when, under the given conditions, practically no biodegradation should occur, and all the biodegradable part of the copolymer was effectively hindered by the polystyrene. Degradation of starch–graft–PMAA copolymers was faster because the copolymer consisted of a biodegradable part and one that was soluble in water. Most of the samples of copolymer had completely disappeared after 14 days of biodegradation in the model river water, while the rest had fully disappeared after 21 days. The degradation mechanism of this copolymer probably included two steps: the attack of microorganisms on the available starch, and, after the degradation of the available starch, the release of chains of poly(methacrylic acid) that were released and soluble in the river water.

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И З В О Д

БИОДЕГРАДАЦИЈА КАЛЕМЉЕНИХ КОПОЛИМЕРА СКРОБА И ПОЛИСТИРЕНА И СКРОБА И ПОЛИ(МЕТАКРИЛНЕ КИСЕЛИНЕ) У РЕЧНОЈ ВОДИ

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У овом раду проучавана је биодеградација калемљених кополимера кукурузног скроба и полистирена и кукурузног скроба и поли(метакрилне киселине) у речној води. Ови кополимери су синтетисани у присуству различитих аминоактиватора. Синтетисани кополимери и продукти биодеградације су карактерисани ФТИР спектроскопијом и СЕМ микроскопијом. Биодеградација је праћена губитком масе узорака, а број микроорганизама Коховом методом. Степен биодеградације обе врсте кополимера расте са временом. Калемљени полимери скроба и поли(метакрилне киселине) потпуно се разграђују за 21 дан, док се полимери на бази скроба и полистирена делимично разграђују након 27 дана (45,8–93,1 % од укупне масе). Разлике у степену биодеградације су последица различите структуре узорака, а постоји и значајна негативна корелација између удела полистирена у кополимеру и степена биодеградације. Степен калемљења полистирена (удео полистирена у кополимеру) који спречава биодеградацију износио је 54 %. На основу експерименталних доказа, предложени су механизми оба биодеградациона процеса и установљени су утицаји количине скроба и синтетске компоненте кополимера на биодеградацију.

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